CLAIMS

1. A method of producing a cloned pig expressing a green fluorescent protein gene, comprising the steps of:
   (a) preparing a nuclear donor cell by culturing a cell line collected from a pig;
   (b) mixing pEFGP-N1 and a lipid component or non-lipid cationic polymer vehicle to form lipid (or cationic polymer)-DNA complexes, and adding the resulting complexes to a culture medium of the nuclear donor cell and further culturing the nuclear donor cell to introduce said GFP gene thereinto and express said GFP gene therein;
   (c) transferring the transfected nuclear donor cell into an enucleated pig recipient oocyte to generate a transgenic nuclear transfer embryo, and activating said nuclear transfer embryo; and
   (d) transplanting the nuclear transfer embryo into a surrogate mother pig to produce live offspring.

2. The method as set forth in claim 1, wherein the lipid component at the step (b) is FuGENE 6 or LipofectAmine Plus.

3. The method as set forth in claim 1, wherein the non-lipid cationic polymer is ExGen 500.

4. A porcine nuclear transfer embryo "SNU-P1 [Porcine NT Embryo]", which is prepared according to the steps (a) to (c) of claim 1, and deposited at KCTC (Korean Collection for Type Cultures) under accesssion number KCTC 10145BP.

5. A cloned pig expressing a green fluorescent protein gene, which is produced from the porcine nuclear transfer embryo "SNU-P1 [Porcine NT Embryo]" of claim 6 by performing the step (d) of claim1.

6. A method of producing a cloned pig having an alpha-1,3-galactosyltransferase gene knocked out, comprising the steps of:

(a) preparing a nuclear donor cell by culturing a somatic cell line collected from a pig;

(b) isolating an alpha-1,3-galactosyltransferase (GT) gene clone from a pig genomic BAC library, and constructing a gene targeting vector using the isolated GT gene, wherein the vector carries a GT gene modified by substituting a portion of a wild-type GT gene with a gene encoding a selectable marker by homologous recombination to suppress expression of a normal GT protein;

(c) mixing the vector with a lipid or non-lipid component to form lipid (or non-lipid)-DNA complexes, and adding the resulting complexes to a culture medium of the nuclear donor cell to allow gene targeting by introducing the recombinant GT gene into the nuclear donor cell;

(d) transferring the nuclear donor cells transfected with the recombinant GT gene into an enucleated pig recipient oocyte to generate a transgenic nuclear transfer embryo, and activating the nuclear transfer embryo; and

(e) transplanting the nuclear transfer embryo into a surrgate mother pig to produce live offspring.

7. The method as set forth in claim 6, wherein the cell line collected from the pig at the step (a) is a fetal fibroblast cell.

8. The method as set forth in claim 6, wherein the gene targeting vector at the step (b) is constructed not to have an exogenous promoter by a promoter trap method.

9. The method as set forth in claim 6, wherein the gene targeting vector at the step (b) comprises a nucleic acid sequence corresponding to a part of intron 8, exon 9 and a part of intron 9 of a GT gene, wherein an AvaI-DraIII fragment of said exon 9 is substituted with a nucleic acid sequence encoding a puromycin-resistant gene linked to a SV40 poly(A) sequence.

10. The method as set forth in claim 6, wherein the lipid component at the step (c) is FuGENE6.

11. A porcine nuclear transfer embryo "SNU-P2 [Porcine NT Embryo]", which is prepared according to the steps (a) to (d) of claim 6, and deposited KCTC (Korean Collection for Type Cultures) under accesssion number KCTC 10146BP.

12. A cloned pig having an alpha-1,3-galactosyltransferase gene knocked out, which is produced from the porcine nuclear transfer embryo "SNU-P2 [Porcine NT Embryo]" of claim 11 by performing the step (e) of claim 6.

13. A vector carrying a nucleic acid sequence corresponding to a part of intron 8, exon 9 and a part of intron 9 of a GT gene, wherein an AvaI-DraIII fragment of said exon 9 is substituted with a nucleic acid sequence encoding a puromycin-resistant gene linked to a SV40 poly(A) sequence.